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Disc Gel Electrophoresis of Minor Milk Proteins

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Suggested Applications

Disc gel polyacrylamide electrophoresis at pH 9.5 (8) and pH 4.5 (10) is a useful tool for resolving milk proteins. In whey the more acidic proteins, β-lactoglobulin, α-lactalbumin, and serum albumin are resolved at pH 9.5 while the more basic proteins and immunoglobulins have relatively slow mobilities under these conditions. These latter proteins can be separated at an acid pH. Disc gel electrophoresis of the whey proteins at alkaline pH will also separate the two genetic polymorphs of β-lactoglobulin designated A and B.

Disc gel electrophoresis of casein at pH 9.5 in the presence of 4 M urea will separate the γ-, β-casein polymorphs A and B as well as β-C casein (5). Apparently, there is no corresponding γ-C. This system will also resolve a number of proteins, TS-, R-, and S-caseins which, like γ-casein, are in casein in relatively small amounts. Although the α_{s1}-casein polymorphs can be separated in this system, α_{s1}-casein is generally typed by a vertical gel slab electrophoretic method (9). Disc gel electrophoresis of casein at pH 4.3 and in the presence of 8 M urea shows good resolution of S-casein and the TS caseins which are resolved to give TS-A and TS-B. The TS-, R-, and S-caseins occur as genetic polymorphs like those of the γ- and β-caseins. For casein samples typed A^2 and B with respect to γ- and β-casein, two corresponding sets of polymorphs are: TS-A^2, S-; and R-, TS-B caseins, respectively. Because of interference from other caseins, the γ- and β-casein polymorphs and also R-casein are difficult to type at this pH. However, the purified samples of γ- and β-casein are well separated under these conditions (2,6). κ-Casein remains at the origin under acid and alkaline conditions of electrophoresis.

Sample Preparation

The casein is separated from skim milk by centrifugation after precipitation by adjusting the milk to pH 4.5. The whey proteins in the supernatant are dialyzed free of salt at 3 C. The casein precipitate is washed several times by centrifugation after resuspending in water and adjusting the pH to 4.6. The casein and whey proteins are recovered by lyophilization.

For disc gel electrophoresis, about .4 mg of the casein or whey proteins are dissolved in .2 ml of the stacking gel solution, and .1 ml sample is applied to the gel columns. With proteins that are relatively pure and for typing the whey fraction for the β-lactoglobulin polymorphs, A and B, only .1 mg sample is needed.

Buffer Composition

Buffer and stock solutions are made according to the Canalco formulations for disc gel electrophoresis with certain modifications (7).

1) *Gel stock solutions for pH 9.5 electrophoresis.** Stock solutions for separation gel: (a) 12 ml of 1 *N* HCl, 9.08 g TRIS, and .057 ml TEMED. Make to 50 ml with water (pH 8.8 to 9.0). (b) 15 g acrylamide, and .4 g MBA. Make to 50 ml with water. (c) .07 g ammonium persulfate. Make to 50 ml with water. Make fresh daily.

Stock solutions for stacking gel:* (d) ~25.6 ml 1 *N* HCl, 2.99 g TRIS, and .23 ml TEMED. Make to 50 ml with water (pH 6.6 to 6.8). (e) 5 g acrylamide, and 1.25 g MBA. Make to 50 ml with water. (f) 2 mg riboflavin.

*Except for the AP solution these gel stock solutions may be stored in amber bottles at 3C for several months.

Make to 50 ml with water.

2) *Electrode buffer pH 9.5 electrophoresis.* 6.0 g TRIS, and 28.8 g glycine. Make to 2 liters with distilled water.

To 1 liter buffer used for the upper electrode add 4 ml of tracking dye (.005% bromophenol blue solution). Buffers are stored at 3 C and may be reused several times.

3) *Gel stock solutions with 4 M urea for pH 9.5 electrophoresis.* Prepare the same as those described above except that 12 g urea is included in the formulation.

4) *Gel stock solutions for pH 4.3 electrophoresis.* Stock solutions for separating gel: (a) 12 ml 2 *N* KOH, ~8.6 ml glacial acetic acid, and 2.0 ml TEMED. Make to 50 ml with water, (pH 4.3). (b)*. (c) .14 g ammonium persulfate. Make to 50 ml with water. Prepare fresh daily.

Stock solutions for stacking gel: (d) 12 ml 2 *N* KOH, ~1.3 ml glacial acetic acid, and .23 ml TEMED. Make to 50 ml with water (pH 6.7). (e)*. (f)*.

5) *Electrode buffer for pH 4.3 electrophoresis.* 62.4 g β-alanine, and ~5 ml glacial acetic acid. Make to 2 liters with distilled water (pH 5.0).

Half of this buffer is used in the lower chamber and half in the upper. These buffers are stored at 3 C and may be reused several times.

6) *Gel stock solutions with 8 M urea for pH 4.3 electrophoresis.* Stock solution for separating gel: (a) 12 ml 2 *N* KOH, 8.6 ml glacial acetic acid, 2.0 ml TEMED, and 24 g urea. Make to 50 ml with glacial acetic acid (pH ~4.6). (b)**. (c)**.

Stock solution for stacking gel: (d)**. (e)**. (f)**.

Gel Composition and Casting

1) *Composition for pH 9.5 electrophoresis.* (a) Separating gel, 7.5% acrylamide. 4 ml stock solution 1a or 3a. 4 ml stock solution 1b or 3b. 8 ml stock solution 1c or 3c.

(b) Stacking gel, 2.5% acrylamide. 1 ml stock solution 1d or 3d. 2 ml stock solution 1c or 3e. 1 ml stock solution 1f or 3f. 4 ml distilled water or 4 *M* urea.

2) *Composition for pH 4.3 electrophoresis.* (a) Separating gel, 7.5% acrylamide. 2 ml stock solution 4a or 6a. 4 ml stock solution 4b or 6b. 2 ml distilled water or 8 *M* urea. 8 ml stock solution 4c. or 6c.

(b) Stacking gel, 2.5% acrylamide. 1 ml stock solution 4d or 6d. 2 ml stock solution 4e or 6e. 1 ml stock solution 4f or 6f. 4 ml distilled water or 8*M* urea.

Gel Casting Procedure

Immediately after mixing the separating gel solution, it is drawn into a syringe, deaerated, and an aliquot, about 1 ml, transferred to glass tubes fitted with rubber stoppers. Either a water or urea solution is then carefully overlayed and polymerization occurs on standing 40 min. The water or urea solution is poured off, a little of the stacking gel solution is added as a rinse, then a layer of stacking gel solution is added and overlayed as before. Photopolymerization of the stacking solution with a fluorescent light takes about 20 min. Finally, the protein sample, .1 ml which fills the tube, is added and allowed to photopolymerize 20 min.

Equipment

Canalco Model 12 disc electrophoresis*** (8,10).

Power Setting and Time

For the first 7 min, 15 mA are applied; then the current is increased to 60 (5mA per tube). A run takes about 35 min for the standard pH 9.5 gels and is stopped when the marker dye approaches the bottom of the gels. Electrophoretic runs at pH 4.3 generally take 60 min. With the pH 9.5 run, most proteins move toward the positive electrode while at pH 4.3 they move toward the negative electrode.

*These solutions are prepared the same as those for pH 9.5. electrophoresis. Storage conditions are also the same.

**Same as those described under 4 b, c, d, e, and f except that 24 g urea is included.

***Reference to brand or firm name does not constitute endorsement by the United States Department of Agriculture over others of a similar nature not mentioned.

The gels are placed in an aniline blue black stain (1 g in 200 ml 7% acetic acid) for 1 h. The gels are then transferred to a 7.5% acetic acid solution. After several changes of acetic acid solutions, the background dye diffuses out, and the stained proteins can be located in the clear gels.

Typical Results

See Fig. 18 to 22.

References

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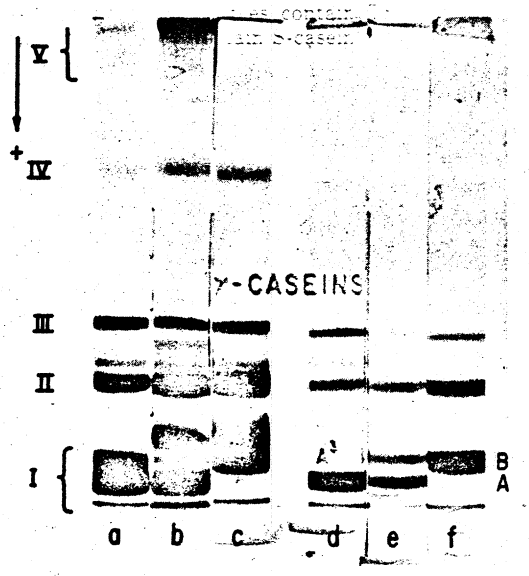

FIG. 18. Disc gel electrophoresis of the whey proteins, standard gels, pH 9.5, I indicates β-lactoglobulin polymorphs. Gels a, b, and c represent β-lactoglobulin types A, AB, and B, respectively, while d, e, and f are corresponding samples, using one-fourth as much protein. Distortions in the β-lactoglobulin zones in gels a, b, and c are due to overloading; II, α-lactalbumin; III, bovine serum albumin; IV, transferrin and possibly other unidentified proteins; and V, immunoglobulins, glycoprotein-a, lactoferrin, lactoperoxidase, and lactollin. (1,3,4)

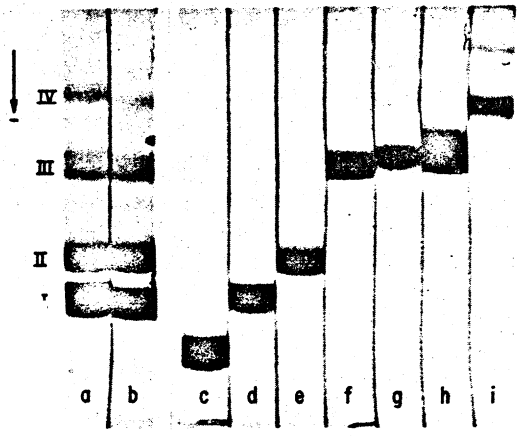

FIG. 19. Disc gel electrophoresis of the whey proteins, acid gels, pH 4.3, I indicates β-lactoglobulin (polymorphs A and B are not resolved); II, α-lactalbumin; III, bovine serum albumin, glycoprotein-a, lactoferrin, and lactoperoxidase; and IV, IgG. Gels a and b are whey protein samples from two individual cows. Gels: c, RNase (isolated from milk); d, β-lactoglobulin; e, α-lactalbumin; f, bovine serum albumin (isolated from milk); g, glycoprotein-a; h, lactoferrin; and i, IgG. (The purified samples of ribonuclease A and β-lactoglobulin were kindly furnished by Mrs. E. W. Bingham and Dr. J. J. Basch.)

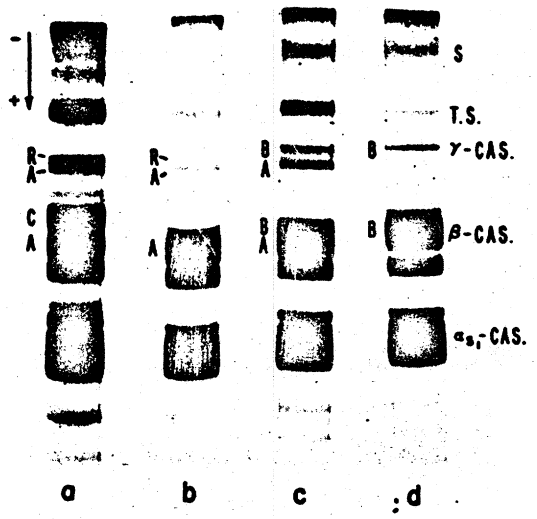

FIG. 20. Disc gel electrophoresis of individual samples of whole casein, pH 9.6, 4 M urea (5). Gels b, c, and d are typed β-, γ-caseins A, AB, and B. Gel a is typed β-casein AC, γ-casein A (γ-casein C is absent). R-casein is indicated in gels a and b. R-casein is present in gel c, but obscured by the γ-AB zones. All samples contain TS-casein while only gels c and d contain S-casein.

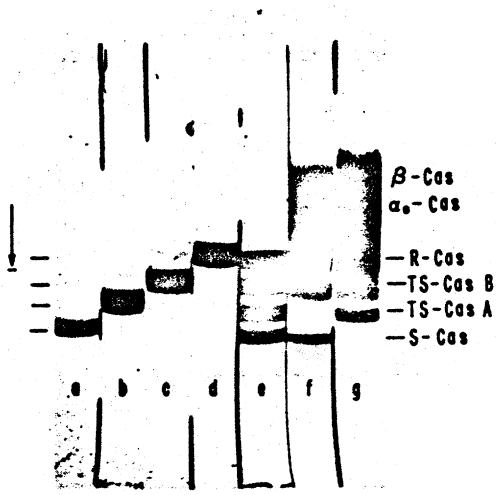

FIG. 21. Disc gel electrophoresis pH 4.3, 8 M urea. Gels a, b, c, and d are the S-, TS-A, TS-B, and R-caseins. Gel e contains all four caseins. Gel f is of casein typed B with respect to β-, γ-casein and shows the S- and TS-B zones (the band corresponding to R is γ-casein B). Gel g is of casein types β-, γ-A^2 and shows the TS-A and R-casein zones.

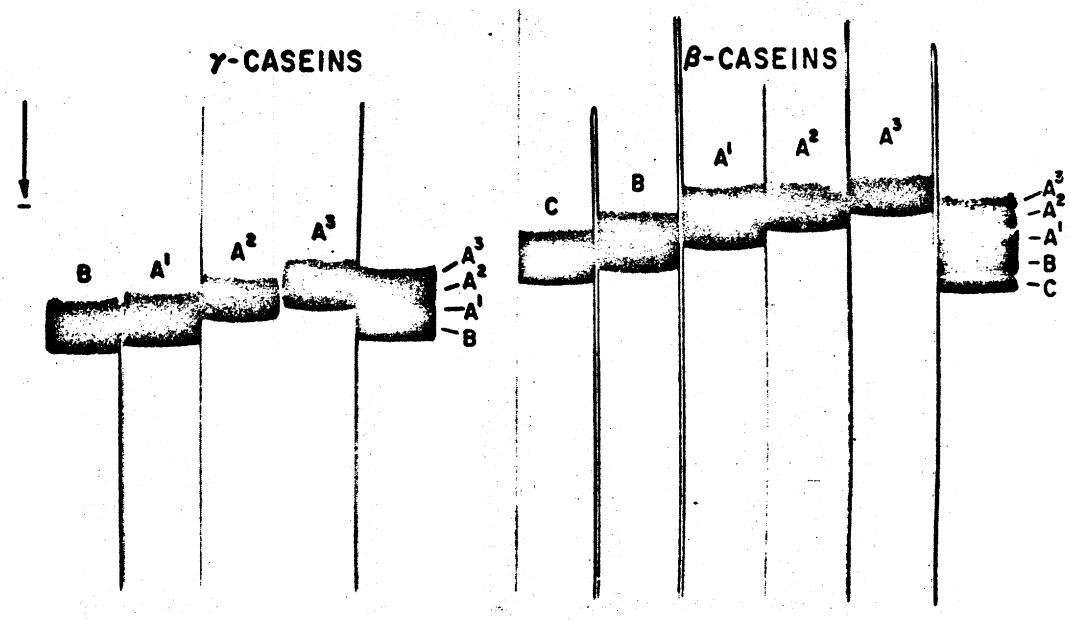

FIG. 22. Disc gel electrophoresis, pH 4.3, 8 M urea of the β- and γ-casein polymorphs.